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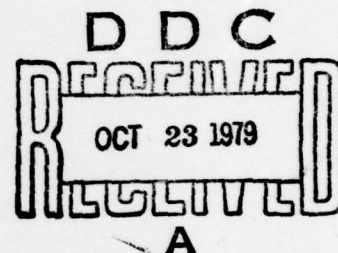
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EVALUATION OF THE HEALTH ASPECTS OF
CERTAIN COMPOUNDS FOUND
IN IRRADIATED BEEF

SUPPLEMENT II
POSSIBLE RADIOLYTIC COMPOUNDS

March 1979



Prepared for

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
DEPARTMENT OF THE ARMY
FORT DETRICK, FREDERICK, MD 21701

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In the absence of direct data, the Committee has utilized findings with model systems and has extrapolated these results to irradiated beef. The uncertainties implicit in such extrapolations are discussed. Rough estimates of beef radiolytic products have been attempted which have permitted speculation of possible toxic effects.

→ There is very little carbohydrate in beef and its radiolytic products should be relatively innocuous in the amounts produced. Few radiolytic products have been identified from the protein component of beef. The bulk of radiolytic products found in beef has come from the fat moiety. Simple triglycerides have been studied extensively and sites of bond scission have been identified. The major products are fatty acids, diglycerides, and diol diesters. The concentrations of these classes have been estimated at approximately 0.5 to 1.0 g per kg irradiated beef. Lesser amounts of many other compounds are possible, including hydrocarbons of varying chain lengths, glycerol, monoglycerides, aldehydes, and ketones. The biological effects of each of these classes have been considered. No evidence is available which indicates a toxicity of any of these compounds at the concentrations anticipated in irradiated beef, although in some cases, inadequate information is available to permit a firm judgment at this time on their possible health effects.

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COMPOUNDS FOUND IN IRRADIATED BEEF.

SUPPLEMENT II

POSSIBLE RADIOLYTIC COMPOUNDS.

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SUMMARY

A number of radiolytic compounds are produced when beef is exposed to sterilizing doses of gamma ray or high energy electron radiation. The identity and concentration of 65 volatile compounds have been determined and their health aspects considered in a previous report. The present report is an attempt to extend these considerations to other possible radiolytic products. In the absence of specific data from irradiated beef, the Committee has utilized the results from model systems of carbohydrates, fats, and proteins to identify possible nonvolatile compounds. Despite the obvious hazard of extrapolating from such simple models to a substance as complex as beef, this method should allow rough estimations of the more likely products.

Simple aliphatic acids and carbonyl-containing compounds may be produced by carbohydrate irradiation, but the small amount of carbohydrate present in beef suggests that the concentrations of such products are low in the irradiated samples. While irradiation of protein is known to produce certain changes few simple compounds attributable to the protein moiety of beef have been detected, although their presence cannot be excluded. Most of the radiolytic compounds thus far detected appear to come from beef fat. Synthetic triglycerides are vulnerable to bond scission at several sites, suggesting a variety of possible radiolytic compounds from the fatty fraction of beef. Rough estimates of the possible concentrations of some of these products in irradiated beef have been attempted by extrapolating data obtained from model lipid systems. The most abundant products appear to be fatty acids, diol diesters, and diglycerides, with significantly lesser amounts of a number of other compounds. The toxicology of these products has been reviewed, wherever possible, but relevant data are lacking or sparse for some of these compounds.

The Committee concludes that many of the radiolytic products in the concentrations estimated to be present, should pose no hazard to consumers of beef irradiated in the described manner. Such products include the individual fatty acids and their simple esters, glycerol, mono- and diglycerides, aldehydes, and aliphatic hydrocarbons. Insufficient data are available to allow judgment of the effects on health of the individual diol diesters and alkylcyclobutanones presumably present. Metabolic and toxicological studies of these compounds are desirable. No evaluation can be made of the other compounds theoretically possible in small amounts, but which have not been demonstrated in irradiated beef or model systems. Because no analysis, however exhaustive, can exclude the possibility of the presence of undetected constituents, no unequivocal demonstration of safety seems possible from consideration of the individual radiolytic products alone. Such analyses, though useful, should be coupled with appropriate animal feeding studies to provide complementary approaches to assure the wholesomeness and safety of irradiated foods.

FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB) provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and opinions of knowledgeable investigators who are actively engaged in work in specific areas of biology and medicine.

A technical report entitled "Evaluation of the Health Aspects of Certain Compounds Found in Irradiated Beef" (LSRO, 1977) was published in August, 1977 by an ad hoc Select Committee with the assistance of the LSRO staff. It reviewed the biological effects of 65 volatile compounds found in irradiated beef. An analysis of the relevant literature on these compounds appearing since this report is included in Supplement I.

Consideration of the radiation chemistry of the major food components suggests that a number of additional compounds could be produced from the exposure of beef to sterilizing doses of ionizing radiation. Most of the compounds discussed in this second supplemental report have not been identified in beef irradiated in the described manner, nor have their concentrations in the beef been determined. Nevertheless, it is believed important to attempt an evaluation of the health aspects of those substances possibly produced during irradiation.

The Select Committee accepts the responsibility for the contents of this report. Appreciation is expressed to Dr. Walter M. Urbain, Special Consultant, for his helpful comments in its preparation. The report was approved by the Select Committee, the Director of LSRO, and the LSRO Advisory Committee composed of representatives of each constituent society of FASEB, under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures, the report was approved and transmitted to the U.S. Army Medical Research and Development Command by the Executive Director, FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of the individual members of its constituent Societies.

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office

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I. INTRODUCTION

For almost two decades, investigators at the Food Sciences Laboratory of the U.S. Army Natick Research and Development Command have been studying the radiolytic products of meats exposed to sterilizing doses of ionizing radiation (Merritt, 1972; Merritt et al., 1959; 1978). Sixty-five compounds have been identified in beef exposed to an average dose of 56 kGy (5.6 Mrads) of gamma ray or electron irradiation at about -30°C. The concentrations ranged from 1 to approximately 700 µg per kg (parts per billion) of irradiated beef. The health aspects of each of these substances were reviewed by a Select Committee of the Life Sciences Research Office of the Federation of American Societies for Experimental Biology (LSRO, 1977, 1979). The Committee concluded that there were no grounds to suspect that these radiolytic products would constitute a health hazard to persons consuming beef irradiated in the described manner.

The 65 compounds identified and discussed in the original reports probably represent only a small portion of those produced by the irradiation of beef. It was their relative volatility which rendered them especially amenable to gas chromatographic and mass spectrometric analysis. However, many other less volatile products are also possible theoretically from the irradiation of a substance as complex as beef.

The major constituents of beef are water, protein, and fat while inorganic salts, carbohydrates, free amino acids, phosphocreatine, and other soluble organic compounds are present in small amounts. Because of the complexity and variability of natural foods, simple compounds of known structure have usually been the substrates for radiolytic studies. Caution must be exercised in extrapolating results with these compounds to more complex substances. Nevertheless such models are useful in illustrating general processes and in providing a basis for speculation about the radiolytic products of various foodstuffs, such as beef.

II. RADIOLYTIC PRODUCTS OF CARBOHYDRATES

A vast literature is available on the radiation chemistry of carbohydrates. A recent review (Dauphin and St. Lebe, 1977) lists almost 400 references on this subject. Depending on the specific carbohydrate irradiated and the conditions of irradiation, hydrogen, carbon dioxide, aldehydes, ketones, acids, and other compounds are formed. Beef contains only about 0.5 percent carbohydrate in the form of muscle glycogen (Swenson, 1977). No report could be found dealing specifically with the radiolysis of glycogen. However, one would expect its radiolytic products to be similar to those of starch or other polysaccharides. Irradiation of polysaccharides leads to their depolymerization and fragmentation into simpler molecules. When wheat starch was irradiated, glucose, maltose, maltotriose, maltotetrose, and maltopentose were all detected in significant amounts, with the di- and trisaccharides predominating (Ananthaswamy, *et al.*, 1970). Small amounts of simpler compounds were also detected after irradiation of wheat starch, mainly formic acid, formaldehyde, acetaldehyde, and glycolaldehyde. However, glycolysis occurs rapidly following death of the animal, so that most of the glycogen originally present in the beef is converted to lactic acid. Radiolysis of lactic acid gives rise to simple one- and two-carbon compounds.

Degradation of any remaining glycogen would also be minimized by the irradiation of beef in vacuo and at low temperatures, both conditions favoring marked reduction of radiolytic activity. Furthermore, many substances, including amino acids and proteins protect carbohydrates against breakdown by radiolysis (Diehl *et al.*, 1978; Phillips, 1972).

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III. RADIOLYTIC PRODUCTS OF PROTEINS

Irradiation produces changes in both the physical and chemical characteristics of proteins, but the changes are not great at doses used in food irradiation. The nature and extent of change depend upon various factors, including the physical structure of the protein, its chemical composition, its native or denatured state, its form during irradiation (dry, wet, frozen), and the presence of other substances. Hydrogen bonds may be disrupted allowing the molecule to unfold; the molecule may undergo dissociation, aggregation, or fragmentation; and individual amino acids may be destroyed. The resulting products are diverse, complex, and difficult to identify or quantify (Urbain, 1977; 1978).

At the amino acid level, the principal radiolytic reactions in oxygen-free solutions are reductive deamination and decarboxylation. These reactions result in the formation of the corresponding organic acids and of amines with one less carbon atom than the original amino acids. Thus, glycine would give rise to methylamine and acetic acid; alanine to ethylamine and propionic acid; etc. In beef, traces of such amines and acids might result from the small amounts of free amino acids present. However, the low irradiation temperature would markedly reduce their production. Taub *et al.* (1978) have shown that freezing reduced the electron-induced ammonia formation from glycine by approximately 90 percent compared with irradiation in the liquid state. In peptides and proteins, only the terminal and diamino- and dicarboxylic amino acids would undergo deamination or decarboxylation. Irradiation in the frozen state would also sharply reduce other radiolytic effects on the protein molecule. This has been demonstrated dramatically with the enzyme pepsin, which retained approximately 95 percent of its original activity when irradiated at temperatures of -30° to -190°C , but only 10 percent when irradiated at room temperature (Bellamy and Lawton, 1954). No amino acid destruction could be detected when beef was irradiated 60 kGy (6.0 Mrad) at -196°C . The individual amino acid content of the beef after irradiation did not differ measurably from the nonirradiated sample (Kauffman and Harlan, 1969), although the analytical methods employed may have been insufficiently sensitive to detect small changes.

The limited data available suggest that only small amounts of simple radiolytic products from protein would result from beef irradiated at low temperatures in the absence of oxygen. Further information on the nature and quantity of such products is desirable.

IV. RADIOLYTIC PRODUCTS OF LIPIDS

The radiolytic products of beef lipids have been studied more thoroughly than those of proteins or carbohydrates. This emphasis does not imply that the radiolysis of lipids is necessarily of greater significance than that of the other beef components, but rather that it has proved more amenable to available analytical techniques. Also, as implied earlier, there has been limited interest in carbohydrate radiolysis because of the small glycogen content of beef. The structural similarity of the triglycerides and their relatively low molecular weights provide attractive models for bond cleavage studies. Studies can be performed on simple synthetic models which are believed to mimic closely the effects of irradiation on more complex natural fats. Many of the products of irradiation are readily identifiable, which allows the investigators to localize the sites of radiolysis and to predict the structure of additional products not yet detected. For these reasons, the radiolytic products of beef lipids are considered here in greater detail than those of carbohydrates and proteins.

Upon exposure to high energy irradiation, lipid molecules are cleaved at a number of sites to produce free radicals, which may then gain or lose hydrogen, or recombine to form a number of new compounds (Nawar, 1977). The most penetrating studies into the mechanism of lipid radiolysis have been those of Nawar and his colleagues, who have utilized a series of simple triglyceride models containing C_4 to C_{18} fatty acids. From these studies, Nawar (1972; 1977) identified five locations (a-e) in the vicinity of each of the carbonyl groups of the triglyceride at which preferential radiolytic cleavage occurs. Scission may also occur on a random basis at the remaining carbon-carbon bonds (site f) in the fatty acid molecule. The general mechanism is summarized in Figure 1.

The most abundant radiolytic products are those formed by the scission of a single bond in the parent compound, followed by abstraction or loss of a hydrogen atom. The number and variety of such potential cleavage products from lipid radiolysis are suggested in Table 1.

The free radicals resulting from these cleavages may recombine to form additional compounds including ketones and diketones, esters, modified triglycerides, glyceryl ether diesters, alkyl propanediol diesters, butanetriol triesters, erythritol tetraesters, and various dimer hydrocarbons (diunsaturated, monounsaturated, saturated). Other radiolytic compounds are possible from multiple cleavages in the same triglyceride molecule or from the further decomposition of products from single bond scission. Hydrogenation, lactone formation, and other reactions may also occur (Nawar, 1977). It is evident that a large number of compounds are

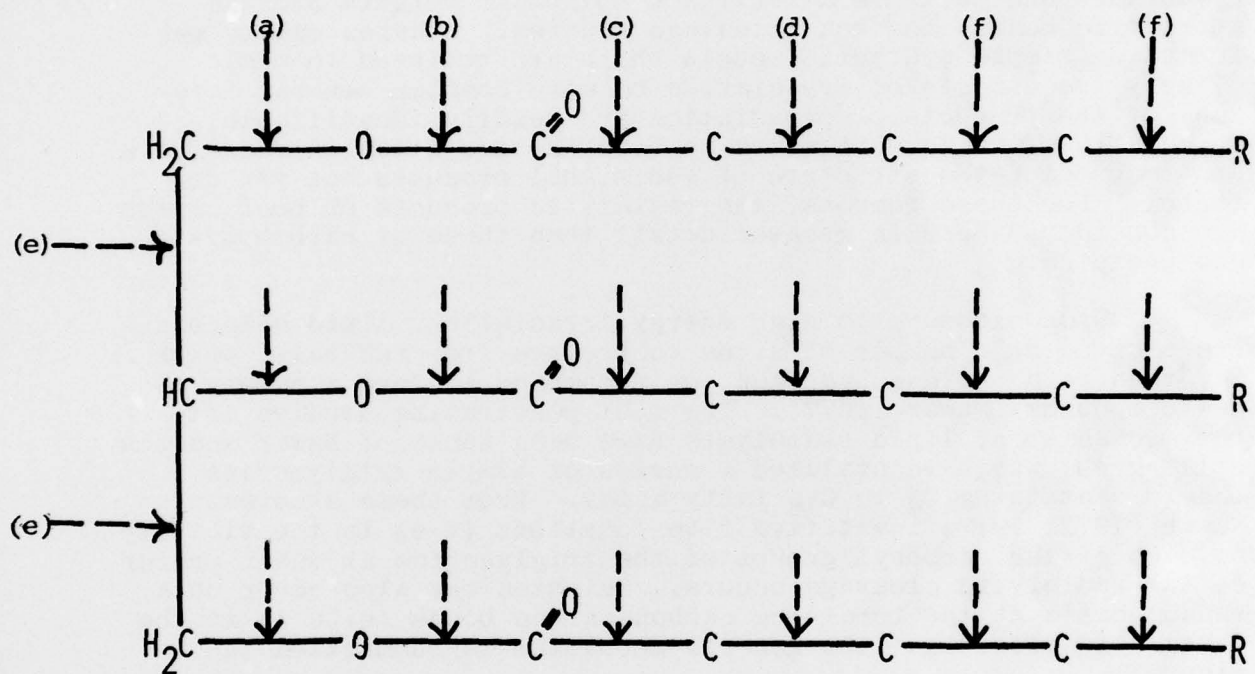


FIGURE 1. Bond scission sites of triglycerides.

Table 1. Lipid radiolytic products.

Site of cleavage (See Figure 1)	Products
(a)	C_n Free fatty acids Propanediol diesters Propenediol diesters
(b)	C_n Aldehydes Alkyl cyclobutanones (C_n) Diglycerides Oxo-alkanediol diesters
(c)	C_{n-1} Hydrocarbons Formyl diglycerides
(d)	C_{n-2} Hydrocarbons Acetyl diglycerides
(e)	Fatty acid methyl esters Ethanediol diesters
(f)	Hydrocarbons (C_{n-4}) Modified triglycerides

theoretically possible, although only a few have been reported under irradiation conditions far more severe than those employed with beef. The total production of these compounds are small compared with those resulting from single bond scission. Nawar (1977) using pure triglycerides found their concentrations to be generally below one part per million. He concluded that they may exist in foods in traces too small to be detected.

In the following sections, attempts will be made to estimate concentrations of various compounds, which from theoretical considerations, might be expected in irradiated beef. Such estimates can be no better than rough approximations, for they are based on extrapolations from simple triglyceride models irradiated at room temperatures. Since irradiation in the frozen state has been shown to reduce radiolytic products (Merritt et al., 1975; Taub et al., 1978), it is believed that the calculated values represent maximal concentrations in the beef, which may in some cases, be substantial overestimations of the actual content. This is illustrated by comparing the concentrations of volatile aliphatic hydrocarbons produced after irradiation of pure triglycerides at 25°C with those in beef irradiated with the same dose at -30°C. The total hydrocarbon production after irradiation of tripalmitin, tristearin, and triolein were 1402, 1562, and 1672 μ moles per kg, respectively (Dubravcic and Nawar, 1968). The corresponding hydrocarbon production in beef irradiated at -30°C was 60 μ moles (LSRO, 1977). Since the fat content of beef was about 25 percent, the hydrocarbon production from the beef fat alone was about 240 μ moles. Thus, hydrocarbon production from the triglyceride models irradiated at room temperature was about six-fold that from fat irradiated in the frozen state. Unfortunately, data are not available to determine whether irradiation of frozen beef results in reductions of comparable magnitude with the other radiolytic compounds under consideration. However, studies with other frozen systems (Bellamy and Lawton, 1954; Taub et al., 1978) suggest a similar tendency.

A. ALIPHATIC HYDROCARBONS

Of the various families of radiolytic compounds, only the aliphatic hydrocarbons have been systematically analyzed. The content of both alkane and alkene members up to 17 carbon atoms in length has been determined in model triglyceride systems (Dubravcic and Nawar, 1968), in pork (Champagne and Nawar, 1969), and in beef (Champagne and Nawar, 1969; Merritt, 1972). These aliphatic hydrocarbons represent approximately 90 percent of the total volatiles analyzed. The health aspects of these compounds have been discussed in previous reports (LSRO, 1977; 1979) and will not be repeated here.

Higher molecular weight aliphatic hydrocarbons from the random recombination of free hydrocarbon radicals (Nawar, 1978), have also been detected after irradiation of model lipid systems. Their presence in irradiated beef is assumed but has not yet been demonstrated. Since the total concentration of such recombination products was much less than of the compounds produced from single bond scission in the model systems, it is believed that the amounts in irradiated beef are probably no more than a few micrograms per kilogram.

No reports could be found concerning biological studies of such long-chain aliphatic hydrocarbons. However, these compounds are widely distributed in natural products and are present as well in oils and waxes employed in pharmaceutical manufacturing and in the processing of foods. Schrier *et al.* (1976) detected virtually the entire alkane and 1-alkene series in grapes, from decane to dotriacontane (C_{32}) and from 1-decene to 1-dotriacontene. Similarly, apple skin contains each of the hydrocarbons from C_9 to C_{31} (Meigh, 1964) with C_{29} predominating. Normal alkanes up to C_{25} have been reported in heated beef (Watanabe and Sato, 1971). Eicosane (C_{20}), docosane (C_{22}), and hexacosane (C_{26}) are present in roasted peanuts (Johnson *et al.*, 1971) and heptacosane (C_{27}) in coffee (Walter and Weidemann, 1969).

B. FATTY ACIDS

Free fatty acids are the most abundant products of triglyceride irradiation (Nawar, 1978). They are produced by cleavage of the acyloxy-methylene bond (site a, Figure 1). The nature and amount of the free acid formed depend upon the fatty acid composition of the triglyceride irradiated. The average composition of beef fat is shown in Table 2 (Swern, 1965).

The quantities of these fatty acids produced during the irradiation of beef have not been reported, but rough estimates are possible from the investigations of LeTellier and Nawar (1972a) with tricaproin. They reported the production of 12.20 μ moles of hexanoic acid per kg of tricaproin irradiated at 60 kGy (6.0 Mrad) and 25°C. This represents approximately 0.2 percent of the hexanoic acid which theoretically could have been produced had the tricaproin been completely hydrolysed. If one assumes the same radiolytic efficiency for other triglycerides, and a beef fat content of 25 percent, the estimated liberation of free fatty acids in beef under similar conditions of irradiation, would range from 12 mg per kg beef for linoleic acid to 234 mg per kg for oleic acid (Table 2) (see footnote* p. 13). Lean beef, with lower fat content, would yield proportionally less fatty acids upon irradiation. These values may overestimate by considerable amounts the actual fatty acid liberation from beef irradiated as described by

TABLE 2. Estimated production of fatty acids by beef irradiation.

Fatty Acid	Structure (Carbons: double bonds)	Beef Fat Composition Percent	Estimated Radiolytic Production (mg/kg beef: ppm)
Myristic	14:0	6.3	30
Palmitic	16:0	27.4	130
Stearic	18:0	14.1	66
Oleic	18:1	49.6	234
Linoleic	18.2	2.5	12

^aAverage composition (Swern, 1965)

^bSee text, p. 13 for calculations employed

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investigators at the U.S. Army Food Sciences Laboratory (Merritt *et al.*, 1978). Beef was irradiated at about -30°C whereas tri-caproin was irradiated at room temperature. As pointed out earlier (Taub *et al.*, 1978), irradiation in the frozen state reduces some radiolytic products by over 90 percent compared with those from liquid solutions.

Simple heating of meat liberates significant amounts of free fatty acids. Hornstein and Crowe (1960) found that each of the constituent fatty acids approximately doubled after heating beef depot fat for 4 hours at 100°C : myristic acid increased from 0.49 to 1.04 mg per g fat; palmitic acid, from 2.24 to 4.91; stearic acid, from 0.96 to 1.37; oleic acid, from 9.24 to 19.74; and linoleic acid, from 0.58 to 1.34. LeTellier and Nawar (1972a) reported that three times as much hexanoic acid was liberated from tricaproin when it was heated at 270°C for 15 hours as when it received 60 kGy (6.0 Mrad) irradiation at 25°C .

Each of the fatty acids of Table 2 is a common constituent of many vegetable and animal lipids regularly consumed in large amounts. The average diet in the United States derives about 40 percent of its calories from fat, or about 100 grams daily. Studies with isotopically labeled triglycerides indicate that approximately 40 percent are hydrolyzed to glycerol and fatty acids during digestion (White *et al.*, 1973). Thus, the daily intake of fatty acids from normal dietary sources is several orders of magnitude greater than that possible from irradiated beef. The Joint FAO/WHO Expert Committee on Food Additives (1974) considered fatty acids to be normal products of fat metabolism and imposed no limits on their acceptable daily intake.

*The following calculations were employed to estimate individual fatty acid production from beef irradiation. On complete hydrolysis, each molecule of tricaproin would release three molecules of hexanoic acid; one millimole tricaproin = 470 mg. Therefore, one kg tricaproin = 2,130 mmoles tricaproin = 6,390 mmoles hexanoic acid. Reported release of hexanoic acid after tricaproin irradiation = 12.20 mmoles/kg. $12.20/6,390 = 0.002$ (radiolytic efficiency).

1 kg trimyristin (mol. wt. 712) = 1,404 mmoles = 4,212 mmoles myristic acid. $4,212 \text{ mmoles} \times 0.002 \text{ (radiolytic efficiency)} = 8.4 \text{ mmoles}$ myristic acid produced/kg trimyristin. Fat in beef is 25 percent; myristic acid is 6.3 percent of fat; $8.4 \text{ mmoles} \times 0.25 \times 0.063 = 0.13 \text{ mmoles} \times 228 \text{ (mol. wt.)} = 30 \text{ mg}$ myristic acid released/kg beef. The production of the other fatty acids was estimated in a similar manner.

Of the fatty acids under consideration in this review, all but linoleic acid can be synthesized by animals. Thus, body stores of myristic, palmitic, stearic, and oleic acids reflect both their dietary intake and their biosynthesis. Linoleic acid, however, must be supplied in the diet as an essential fatty acid. Its exact role in the body is still uncertain, but its deficiency in the rat results in impaired growth, eczematous dermatitis, and impairment of reproduction (Alfin-Slater and Aftergood, 1973). Relatively large amounts can be fed without apparent harm. Swell et al. (1962) maintained 16 healthy subjects for 1 year on a high vegetable fat diet in which 22 percent of the fat calories was obtained from linoleic acid. No significant change in weights of the subjects was noted and no adverse effects were reported during the experimental period. The average serum cholesterol levels dropped from 293 to 230 mg per dl during this period. In another experiment, men were fed a diet rich in unsaturated fats for 5 years with no reported ill effects. During this period the linoleic acid of the adipose tissue rose from 11 to 32 percent of the total fatty acids (Dayton et al., 1966).

C. FATTY ACID ESTERS

Although traces of other alkyl esters of the fatty acids may be present, available evidence suggests that the methyl esters predominate. LeTellier and Nawar (1972a) detected methyl hexanoate but no other fatty acid ester, after the irradiation of tricaproin. Presumably, other esters, if present, were in concentrations below the sensitivity of the analytical methods employed. Approximately 50 mg (0.39 mmoles) of methyl hexanoate were formed per kg of tricaproin irradiated with 60.0 kGy (6.0 Mrad). Extrapolating from these limited data, the following rough estimates can be made of the methyl esters in irradiated beef: 6.5, 3.6, 1.8, 0.8, and 0.3 mg per kg for methyl oleate, palmitate, stearate, myristate, and linoleate, respectively. As pointed out earlier (p. 13), these values are apt to be overestimates because the frozen state of the beef during irradiation would reduce the amounts of radiolytic products.

The methyl and ethyl esters of each of these fatty acids have been detected in a number of natural foods (Van Straten, 1977) although their concentrations were not reported. In addition, methyl myristate is used as a flavoring agent in candy at concentrations up to 2.4 mg per kg and in beverages, ice cream, baked goods, gelatins, and puddings at levels of 0.25 to 0.50 mg per kg (Hall and Oser, 1965).

The Committee knows of no reports which specifically address the fate of these methyl esters in the body. Lipases hydrolyze acylglycerides, while simple, nonspecific esterases catalyze the scission and synthesis of esters of lower alcohols and fatty acids (Stolz, 1956). Also, a number of methylation and

demethylation reactions have been reported in normal metabolic processes (Handler and Perlzweig, 1945). Presumably the methyl esters of fatty acids would be subject to similar enzymatic action. No reports could be found of studies on the acute toxicity of these compounds. However, Alfin-Slater *et al.* (1965) studied effects of several methyl esters of fatty acids in short-term feeding experiments. Weanling male and female rats (USC strain), maintained on a fat-free diet were given 100 mg per day (about 2.0 g per kg at start of the experiment) of various methyl esters for 12 weeks. Rats receiving methyl myristate, palmitate, or stearate gained slightly less weight than the control animals, while those supplemented with methyl oleate or linoleate, gained more than the controls during the experimental period. Methyl oleate, but not the other methyl esters, raised the level of hepatic cholesterol esters above that obtained with the unsupplemented fat-free diet. Smith *et al.* (1960) fed rabbits a diet supplemented with 10 percent methyl linoleate (about 3 g per kg body weight) three times weekly for an unspecified period. There was no significant alteration in the tissue cholesterol levels. No deleterious effects were reported.

D. GLYCEROL

Only very small amounts of glycerol would be expected from the irradiation of fats, for three cleavages on the same triglyceride molecule would be required for its production. Most radiolytic products result from a single bond scission and the probability of two or more cleavages on the same molecule is low (Nawar, 1978).

Relatively large amounts of glycerol are absorbed from the normal diet. It has been estimated that about 40 percent of the ingested triglycerides are hydrolyzed to glycerol and fatty acids in the gastrointestinal tract (White *et al.*, 1973) representing a daily intake of glycerol of about 4 g per day. In addition to its presence in natural fats, glycerol is also employed in food processing and is listed as GRAS (generally recognized as safe) in the Code of Federal Regulations [21 CFR 182.1320] (Office of Federal Register, 1977). The glycerol produced from radiolytic breakdown of triglycerides would be only an extremely small fraction of the amount absorbed from normal diets.

Glycerol is readily metabolized in the body. Gidez and Karnovsky (1954) administered ^{14}C -glycerol to rats intraperitoneally, intravenously, or intragastrically and demonstrated the incorporation of labeled carbon into blood glucose, liver glycogen, and tissue lipids. Only 1 to 5 percent of the administered radioactivity could be recovered in the urine and feces. About 40 percent of glycerol was oxidized within a 6 h period to carbon

dioxide. Radioactivity was found in the lipids of most tissues examined, including the brain. The acute oral toxicity of glycerol is extremely low, with reported LD₅₀ values (g per kg body weight) ranging from 7.8 to 10.0 for guinea pigs (Hine et al., 1953; Smyth et al., 1941); 19.3 to 31.5 for mice (Fischer et al., 1949; Latven and Molitor, 1939); and 27.2 to 48.5 for rats (Fischer et al., 1949; Hine et al., 1953).

Long-term feeding of glycerol to rats (Atlas Chemical Industries, 1969; Hine et al., 1953), or dogs (Food and Drug Research Laboratories, 1962) caused no treatment-related adverse effects. Male and female rats (Long-Evans strain) were maintained on diets containing 5 or 10 percent glycerol (about 5 or 10 g per kg body weight daily) for 2 years, or 20 percent (about 20 g per kg per day) for 1 year (Hine et al., 1953). No significant biochemical or pathological changes were detected. A similar study with Sprague-Dawley rats, also fed 5, 10, or 20 percent glycerol for 2 years produced no ill-effects (Atlas Chemical Industries, 1969). Dogs fed diets containing up to 20 percent glycerol (about 5 g per kg per day) revealed no pathological changes (Food and Drug Research Laboratories, 1962).

Six generations of rats were reared on diets containing 10 percent glycerol without significant influence on growth or reproduction (Guerrant et al., 1947). Administration of 1 g per kg body weight to pregnant mice or rats (days 6 through 15 of gestation) produced no significant changes from control animals in maternal or fetal survival or in the incidence of offspring abnormalities (Food and Drug Research Laboratories, 1973). Johnson et al. (1933) fed 110 g of glycerol daily (from 1.3 to 2.2 g per kg body weight) to 14 young volunteers for 50 days. No ill-effects were noted.

Glycerol has been administered both orally and parenterally in patients to reduce cerebral edema, ocular tension, or cerebrospinal fluid pressure (Tourtellotte et al., 1972). Concentrations up to 40 percent have been used intravenously without causing hemolysis.

E. MONOGLYCERIDES

Two cleavages of the same triglyceride molecule are necessary to form a monoglyceride, and since this would occur only infrequently, the amount resulting from the radiolysis of beef fat should be small. Of the five possible monoglycerides of beef, those of oleic and palmitic acids are most likely to be formed because of their greater abundance in beef fat. These monoglycerides as well as those with other fatty acids are produced during normal digestive processes in far greater amounts than could be produced by the radiolysis of beef fat. Mattson and Volpenhein (1964)

found that approximately three-quarters of triglycerides ingested by the rat were hydrolyzed in the intestinal lumen to 2-monoglycerides. The monoglycerides entered the intestinal cells intact, and were reesterified to triglycerides. Kayden *et al.* (1967) fed five men doubly-labeled monoglycerides and detected the radiolabel in their lymph. They concluded that the 2-monoglyceride pathway appears to be the major route of fat absorption for man during normal digestion and absorption of dietary triglyceride.

In addition to the amounts arising from normal fat digestion in the gastrointestinal tract, monoglycerides are also consumed from many natural edible oils and from various commercial preparations. Natural oils may contain up to 1 percent of monoglycerides which may increase appreciably during normal cooking processes. Approximately 0.5 percent of monoglycerides in lard and detectable amounts in bread have been reported (Kuhrt *et al.*, 1952). A number of synthetic monoglycerides are also used extensively by the food industry, primarily as emulsifying agents. Monoglycerides of edible fats and oils, as well as certain synthetic analogues have been accorded GRAS status by the Food and Drug Administration [21 CFR 182.4521] (Office of the Federal Register, 1977).

Mattson *et al.* (1951) found monoglycerides to be nutritionally equivalent to di- and triglycerides of corresponding fatty acids. Weanling rats fed pure monoglycerides at a 25 percent level (about 25 g per kg per day) for 10 weeks showed no gross or microscopic pathology. Similarly, hamsters fed 5 or 15 percent of glycerylmonostearate for 22 to 28 weeks revealed no significant differences from controls in growth, food intake, or tissue changes (Orten and Dajani, 1957).

F. DIGLYCERIDES

Although some triglyceride molecules may be subjected to double or triple cleavages during irradiation, to produce monoglycerides or glycerol, respectively, they are more likely to undergo a single bond scission and leave a diglyceride residue. LeTellier and Nawar (1972a) reported the production of 2.95 mmoles (761 mg) dicaproyl per kg of tricaproyl irradiated at 25°C and with 60 kGy (6.0 Mrad). It was the most abundant radiolytic product identified apart from the fatty acid (caproic acid) and propanediol diester. Assuming beef to contain 25 percent fat, the diglyceride production under comparable irradiation conditions would be less than 200 mg per kg beef. Irradiation in the frozen state should yield substantially less diglyceride. Fifteen different diglycerides are possible from the five major fatty acids comprising beef fat, exclusive of positional isomers. The actual distribution of these diglycerides is unknown, but those containing oleic and palmitic acids would be expected to predominate.

Diglycerides are normally produced during digestion of fats and are readily utilized by the body. They are also used extensively as emulsifying agents by the food industry and are approved by the Food and Drug Administration [21 CFR 182.4505] (Office of the Federal Register, 1977). Many of these preparations are synthesized by direct esterification of glycerol with fatty acids, or by partial hydrolysis of natural triglycerides. The resulting mixture may contain both mono- and diglycerides as well as some glycerol, triglycerides, and fatty acids.

Relatively few reports could be found on biological aspects of natural diglycerides, presumably because their effects are assumed to be similar to the parent triglyceride. Mattson *et al.* (1951) fed mono-, di-, and triglycerides containing the same fatty acids to weanling rats as 25 percent of a synthetic diet. Except for slight inherent differences in caloric values, the three lipids were nutritionally equivalent. Harris and Sherman (1954) confirmed these findings with rats maintained for 70 days on an equicaloric, restricted, pair-feeding basis. Male weanling rats were fed mono-, di-, or triglycerides and various mixtures of these as 15 and 25 percent of a synthetic-type diet. All forms provided equivalent nutritive value.

G. MODIFIED GLYCERIDES

Scission of carbon-carbon bonds (sites d,f of Figure 1) of the fatty acid moieties of the triglycerides would liberate the various hydrocarbons already discussed, together with a family of diglyceride esters of varying lengths. Only the simplest of these esters, formyl- and acetyldiglycerides have been detected after irradiation of triglyceride models.

LeTellier and Nawar (1972a) reported 0.79 mM of acetyl dicaproin per kg was produced upon irradiation of tricaproin at 60 kGy (6.0 Mrad) at 17°C. If the same degree of radiolysis occurred in beef with a 25 percent fat content, approximately 100 mg acetyl diglycerides per kg beef would be produced. The concentration of formyl dicaproin was too low to permit quantitation. No information is available on the biological fate of these compounds, although hydrolysis to diglyceride and formic or acetic acid would seem likely.

H. ALDEHYDES

Cleavage of the fatty acid chain at the acyl-oxy bond (site b of Figure 1) gives rise predominantly to aldehydes with the same number of carbon atoms as the parent fatty acid. From beef fat, the following aldehydes would be anticipated: tetra-

decanal (myristaldehyde), hexadecanal (palmitaldehyde), octadecanal (stearaldehyde), octadecenal (oleylaldehyde) and octadecadienal (linolylaldehyde). Each of these aldehydes, except for octadecadienal, was detected in irradiated beef, and was discussed in the original report (LSRO, 1977).

In the absence of air, irradiation of lipid produces only small amounts of aldehydes and ketones. The amount produced should reflect roughly the content of the fatty acid composition of the irradiated fat. Thus, oleic acid, which comprises approximately half of the fatty acids in beef fat yielded 398 μg of oleylaldehyde per kg beef after irradiation, and palmitic acid, the next most abundant acid, yielded 127 μg of palmitylaldehyde per kg beef (LSRO, 1977). Linoleic acid, comprising only 2.5 percent of the total fatty acids in beef fat would be expected to provide approximately 10 to 20 μg of the corresponding aldehyde per kg beef. However, although this estimated concentration of octadecadienal was within the sensitivity range of the analytical procedures employed, none was detected after beef irradiation in vacuo (LSRO, 1977).

No information on the metabolism or biological action of octadecadienal could be found. Aldehydes as a class are readily oxidized in the animal body to the corresponding acid and are normally converted by beta-oxidation to carbon dioxide and water (Williams, 1959). Alternatively, some aldehydes and ketones, especially of xenobiotic origin, may be reduced to their alcohols and metabolized accordingly (Kessler and Ferrell, 1974). Which, if either, of these pathways is followed by octadecadienal is not known.

Monty et al. (1961) reported the presence of small amounts of 2, 4, 5, 8, and 9 carbon aldehydes as "minor compounds" when ground meat was irradiated in a nitrogen atmosphere with 46 or 93 kGy (4.6 or 9.3 Mrad) at 20°C. Neither the exact structures of these aldehydes nor their concentrations were determined. It is assumed that they were n-aliphatic monocarbonyls. None of these aldehydes was detected by Merritt et al. (1972) with 56 kGy (5.6 Mrad) irradiation at about -30°C presumably because of the markedly lower temperature employed. Each of these lower aldehydes is found naturally in many foods, including meats (Van Straten, 1977). All are widely used as flavoring substances and are generally recognized as safe by the Flavoring Extract Manufacturers Association (Hall and Oser, 1965). The Council of Europe (1974) also includes them as artificial flavoring substances which may be added to foodstuffs without hazard to health. The Council has established an acceptable human daily intake for each of these aldehydes at 1 mg per kg body weight.

I. KETONES

Several aliphatic ketones have been reported after irradiation of synthetic triglycerides or meats at room temperature. Monty *et al.* (1961) detected 3 to 9 carbon as well as "long-chain" ketones in beef, pork and chicken irradiated under nitrogen at 46 and 93 kGy (4.6 and 9.3 Mrad). The individual ketones were not identified but were assumed to be n-alkan-2-ones. The amounts of ketones produced were not determined but the total carbonyl content (aldehydes plus ketones) in beef with 93 kGy (9.3 Mrad) was 1.01 mMoles (approximately 100 mg per kg), with more than half consisting of four to six carbon aldehydes and ketones. Three aliphatic ketones (2-pentanone, 3-hexanone, and 4-heptanone) were detected among the radiolytic products of tributyrin subjected to 500 kGy (50 Mrad) radiation *in vacuo* at room temperature (Meidani *et al.*, 1977). These simple aliphatic ketones are flavorful compounds found normally in many foods (Van Straten, 1977) and often employed as artificial flavoring agents (Council of Europe, 1974; Hall and Oser, 1965).

Merritt (1972) was unable to detect these volatile ketones in beef irradiated in the frozen state. He found only acetone and 2-butanone when beef was subjected to 56 kGy (5.6 Mrad) irradiation at -30°C *in vacuo*. The production of cyclic ketones has also been reported after the irradiation of triglyceride models. LeTellier and Nawar (1972b) detected 2-alkylcyclobutanones of the same carbon number as the constituent fatty acid. These investigators (1972a) reported that 0.96 mM of 2-ethyl cyclobutanone per kg triglyceride (about 94 mg) resulted from the irradiation (60 kGy; 6.0 Mrad) of tricaproin at 17°C. Assuming a fat content in beef of 25 percent and the production of comparable amounts of 2-alkylcyclobutanones from the individual fatty acids in the beef fat, a production of 50 to 60 mg of these cyclic ketones per kg beef is possible upon irradiation at 17°C. The amounts resulting from beef irradiated in the frozen state are unknown but would presumably be significantly lower.

No information is available on the health aspects of alkylcyclobutanones.

J. DIOL DIESTERS

A number of diol diesters are possible from the radiolysis of fat. As shown in Figure 1 (site e), cleavage between the primary and secondary carbons of the glyceryl skeleton will produce ethanediol diesters; cleavage at the acyloxymethene bonds of the triglyceride (site a) results in propanediol diesters, which can be converted to propenediol diesters by the subsequent loss of hydrogen; cleavage at the acyloxy bond of the fatty acids (site b), followed by a loss of hydrogen would produce oxopropanediol diesters.

Each of these diol diesters was detected when tricaproin was irradiated in vacuo at 60 kGy (6.0 Mrad) at 17°C. By far the most abundant were the propanediol dicaproates (1,2 and 1,3) which together comprised about 85 percent of the total. Most of the remaining 15 percent consisted of ethane- and propenediol dicaproates. Traces of the oxo-derivatives (1-oxo-2,3 and 2-oxo-1,3 propane dicaproates) were also detected, but in amounts too small to permit quantitation.

An attempt has been made to estimate the amounts of diol diesters produced by the irradiation of beef. The only quantitative data available are those of LeTellier and Nawar (1972) on tricaproin irradiated at 60 kGy (6.0 Mrad) and 17°C. In extrapolating these values to irradiated beef (Table 3), it was assumed that beef contained 25 percent fat and that the fat consisted only of triolein. These simplifying assumptions do not alter significantly the estimation of total diol diesters although it provides no information on the content of the individual compounds. The five fatty acids comprising the bulk of the beef fat could produce 15 different diester combinations. The concentration of each diester would be a reflection of the relative fatty acid composition of the beef fat.

The calculations suggest that the total diol diester production would be approximately 1 g per kg beef irradiated at 17°C. As indicated earlier, irradiation as performed at -30°C should yield substantially lesser amounts.

The presence of diol compounds in lipid material was first reported in plant seeds in 1961 (Ukita and Tanimura, 1961). They were later detected in microorganisms (Asselineau, 1961; DeMarteauginsberg and Miguel, 1962), and in mammalian tissue, when ethylene glycol was identified in hydrolysates of beef lung lipids (Carter et al., 1963). Since then, diol compounds have been found in the lipids of bacteria, yeast, plant seeds, invertebrates, and mammals (Bergelson, 1973). The laboratories of Bergelson in the Soviet Union and of Baumann and Schmid in the United States have been especially active in the isolation, structure determination, and biosynthesis of these substances. Bergelson and coworkers detected derivatives of 1,2-ethanediol as well as of 1,2-propanediol, 1,3-propanediol, 1,3-butanediol, 2,3-butanediol, and 1,4-butanediol in a number of bacteria, animal, and plant cells (Bergelson et al., 1964; Vaver et al., 1971). That the diol lipids may have a biological role analogous to that of triglycerides was suggested by the recovery of ethylene glycol dipalmitate from regenerating rat liver (Vaver et al., 1969). Subsequently, Vaver et al. (1972) identified three ethylene glycol diesters of heptadecanoic, heptadecenoic, stearic, and oleic acids from yeasts grown on heptadecane, indicating biosynthetic processes similar to those of triglycerides. Ethane-, propane-, and butanediols can also form the backbone of phospholipids as well as of glycolipids. Baumann et al. (1975) found approximately 350 µg of diol lipids per g of rat

TABLE 3. Estimated radiolytic production of diol diesters.

Compound	From tricaprion ^a mM/kg	From beef fat ^b mg/kg	From beef ^c mg/kg
1,2-Ethane diol diester	0.12	73	18
1,2-Propane diol diester	3.24	1967	492
1,3-Propane diol diester	2.21	1341	335
1,3-Propene diol diester	0.45	246	61
2,3-Propene diol diester	0.32	194	49
TOTALS	6.34	3821	955

^aModified from LeTellier and Nawar (1972a)

^bCalculated as 100 percent triolein

^cAssuming 25 percent fat

liver. The concentration in other mammalian tissues was not determined. About two-thirds of the total were 1,2-ethanediol derivatives, with the remaining third approximately evenly divided among the derivatives of 1,2-propanediol; 1,3-propanediol; and 1,3-butanediol.

Little is known of the synthesis, metabolism, or physiological role of the naturally occurring diol lipids. Schmid and coworkers have published a series of reports on some aspects and have shown that the rat brain can incorporate long-chain 1,2-alkanediols into diol phospholipids (Chang and Schmid, 1973; Schmid *et al.*, 1975) as well as into glycerophosphatides (Chang and Schmid, 1975).

Diol-derived lecithin analogues possess strong hemolytic actions (Baer, 1953; Reman *et al.*, 1969). Bergelson (1973) speculated that in the low concentrations found in mammalian tissues, such diol lecithins may increase the permeability of cell membranes or otherwise modify their properties.

Toxicity data (Table 4) could be found only for the simple diols. Similar data are not available for the diol diesters presumably present in irradiated beef nor for various derivatives which might be present in small amounts.

1,3-Butanediol (BD) and 1,2-propanediol (PD) have been studied as synthetic sources of dietary calories. Mehlman and colleagues (1970) have shown that the addition of BD to rat diets increased the activity of liver and kidney gluconeogenic enzymes and the formation of ketones. Kies *et al.* (1973) substituted 15 g daily for an isocaloric amount of starch in the diet of 12 human subjects for 14 days. Apart from a lowering of the blood glucose, no alterations of blood chemistry or cytology were noted. The investigators concluded that BD may be useful in diabetic diets. Emmanuel and Nahapetian (1975) fed diets containing 5 or 10 percent BD, or 5 percent PD to sheep for 6 weeks. PD had no effect on blood ketones, but increased serum glucose slightly. BD, on the other hand, increased ketone bodies and reduced blood glucose slightly. They concluded that BD and PD can replace at least part of the readily available carbohydrate without producing adverse effects on animal performance.

Table 4. Oral toxicity of simple diols (NIOSH, 1977).

Compound	Animal	LD ₅₀ (g/kg)
1,2-Ethanediol	Rat	5.8
	Mouse	7.5
	Cat	2.0
	Guinea pig	6.6
1,2-Propanediol	Rat	21.0
	Mouse	24.0
	Dog	22.0
	Guinea pig	19.0
1,3-Butanediol	Rat	23.0
	Guinea pig	11.0
1,4-Butanediol	Rat	1.5
	Mouse	2.1
	Rabbit	2.5
	Guinea pig	1.2

V. DISCUSSION

The availability of sensitive analytical techniques has simplified the determination of volatile compounds in various media. Consequently, far more data are now available on such volatile radiolytic products of foods and model systems than on non-volatile compounds, for which methods of comparable simplicity and sensitivity are lacking. Attempts to determine nonvolatile products have focused on simple model systems rather than on foods, so that the sites of radiolytic action could be more easily identified. Thus, in the absence of direct data on these products in irradiated beef, it has been necessary to extrapolate the findings from relatively few experiments in which substances of known structures have been used. Such extrapolations have obvious shortcomings, for not only is beef a far more complex substance than the models utilized, but other variables also influence the kind and amount of radiolytic products. Temperature, radiation dose, fat content and composition, physical status, ambient atmosphere and other factors may affect the nature and concentrations of the compounds produced. The Select Committee is well aware of these objections but believes nevertheless, that these extrapolations can provide useful information on the nature of the compounds produced in irradiated beef, and can allow a rough approximation of their concentrations.

Most emphasis in this report has been placed on the radiolytic products of the fatty moiety of beef. Considerable data are available on the radiolysis of various synthetic triglycerides, which provide a useful base for comparison with beef irradiation. Also, it has been shown that most of the volatile compounds identified after beef irradiation arose from the fatty components. Animal muscle contains only 1 percent or less of carbohydrates, mostly glycogen, which is rapidly converted to lactic acid upon death of the animal. The radiolytic products from this source would be small in amount and would consist largely of compounds containing one or two carbons found in commonly consumed foods or normally produced in the body. The primary structure of proteins can be affected by irradiation and this action is reflected by changes in their physical characteristics or biological function. A few volatile compounds, presumably arising from amino acids, have been detected in irradiated beef, but data are lacking which would permit estimations of the nature and concentrations of other simple products from beef proteins irradiated under the conditions now employed.

The major radiolytic products from synthetic triglycerides are free fatty acids, diol diesters, and diglycerides. Extrapolation of data from these model systems to beef suggests that about 1.0 g of the various diol diesters, 500 mg of fatty acids, and 200 mg of diglycerides per kg beef might be produced after irradiation. As pointed out earlier, there is evidence that

these, as well as other values obtained by extrapolation from triglyceride models may seriously overestimate the amount actually produced when beef is irradiated in a frozen state. Free fatty acids and diglycerides occur naturally in many foodstuffs, are normal digestive and metabolic products, and are authorized additions to food for desirable technical effects. The amounts produced by irradiation would be small compared with their intakes from other sources and would not be expected to have adverse health effects.

Less is known of the diol diesters, the other major product of fat irradiation. There is evidence that they are natural constituents of mammalian tissues and that they are produced in significant quantities during normal heating processes of fatty foods. Thus, tricaproin heated at 270°C for 15 hours produced ten times as much ethane dioldiester as did 60 kGy (6.0 Mrad) irradiation at 25°C. However, metabolic and toxicity data are not available on the individual members of this family, precluding any firm judgment of their possible health effects.

Based on studies with tricaproin, the only other radiolytic products present in detectable amounts were esters of fatty acids and diglycerides, aldehydes corresponding to the fatty acid components of the fat, and alkylcyclobutanones. Various aldehydes have been detected in irradiated beef in concentrations less than one part per million and were considered in the previous report to pose no hazard in these amounts to the consumer. It is believed that the esters would be hydrolyzed by gastrointestinal and tissue esterases to yield harmless levels of fatty acids and diglycerides. Nothing is known of the fate and toxicity of the alkylcyclobutanones, so no judgment can be rendered on their possible health effects.

Although relatively few radiolytic products have been detected in irradiated beef or model systems, and even fewer have been determined quantitatively, many more are possible theoretically. Many of such putative products are found in natural foods, added during food processing, produced during cooking, or present in the body as normal metabolites. As Nawar (1977) has pointed out, the nature of decomposition products formed by irradiation and heat treatment are quite similar, with many more of such compounds identified in heated or thermally oxidized, than in irradiated samples.

It is not possible to compile a complete inventory of all the components of natural foodstuffs nor of all conceivable irradiation products. The possible presence of undetected substances can never be excluded. For this reason, it is desirable to complement such chemical studies with animal feeding experiments in which the effects of irradiated and of nonirradiated beef are compared. Such experiments provide an added approach to determine wholesomeness and safety of irradiated foods.

VI. CONCLUSIONS

In the light of the foregoing considerations, the Committee concludes that:

1. Many of the radiolytic products in the concentrations estimated to be present appear to pose no hazards to consumers of beef irradiated in the described manner. Such products include the individual fatty acids and their simple esters, glycerol, mono- and diglycerides, diglyceride esters, aldehydes, and aliphatic hydrocarbons.

2. Insufficient data are available to allow judgment of the effects on health of the individual diol diesters and alkylcyclobutanones presumably present. Metabolic and toxicological studies of these compounds are desirable.

3. No evaluation can be made of other compounds theoretically possible in small amounts, but which have not been demonstrated in irradiated beef or model systems. Because no analysis, however exhaustive, can exclude the possibility of the presence of such theoretical but undetected constituents, no unequivocal demonstration of safety seems possible from consideration of individual radiolytic products alone.

4. It is desirable to couple chemical studies as described in this report with suitable animal feeding studies to provide complementary approaches to ensure the wholesomeness and safety of irradiated foods.

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